PB81-129892

Effects of Waste Oil on the Survival and Reproduction of the American Flagfish 'Jordanella floridae'

(U.S.) Environmental Research Lab.-Duluth, MN

1980

U.S. Department of Commerce
National Technical Information Service
NTIS.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/J-80-191	Journal Article	

4. TITLE AND SUBTITLE
Effects of Waste Oil on the Survival and Reproduction of the American Flagfish, Jordanella floridae

5. REPORT DATE

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
Steven F. Hedtke and Frank A. Puglisi

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
U.S. Environmental Protection Agency
Environmental Research Laboratory-Duluth
6201 Congdon Boulevard
Duluth, MN 55804

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
Same as above

13. TYPE OF REPORT AND PERIOD COVERED
Journal article

14. SPONSORING AGENCY CODE
EPA-600/03

15. SUPPLEMENTARY NOTES
REFERENCE: Can. J. Fish. Aquat. Sci. 37: 757-764 (1980).

16. ABSTRACT

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17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	9
	20. SECURITY CLASS *(This page)*	22. PRICE
	UNCLASSIFIED	

PB81-129892

EPA-600/J-80-191
Journal Article

Reprinted from

Canadian Journal of Fisheries and Aquatic Sciences

Réimpression du

Journal canadien des sciences halieutiques et aquatiques

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Volume 37 • Number 5 • 1980

Pages 757–764

Government of Canada Fisheries and Oceans • Gouvernement du Canada Pêches et Océans

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

Effects of Waste Oil on the Survival and Reproduction of the American Flagfish, *Jordanella floridae*

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HEDTKE, S. F., AND F. A. PUGLISI. 1980. Effects of waste oil on the survival and reproduction of the American flagfish, *Jordanella floridae*. Can. J. Fish. Aquat. Sci. 37: 757–764.

Short-term lethality tests, tests on the 30-d survival of 1-d-old larvae, and a life cycle test showed that used crankcase oil adversely affected the survival and reproduction of the American flagfish, *Jordanella floridae*. Four-day LC_{50}'s for juveniles in static and flow-through tests were 36 200 μL/L and 9500 μL/L, respectively, for the water-soluble fraction (wsf) of a 10% oil–water mixture and 485 μL/L and 82.7 μL/L, respectively, for oil–water emulsions. The general descriptive characteristics of the test oil and the hydrocarbon composition and concentration of 13 metals were determined for the oil and its water-soluble fraction. The life cycle test began with embryos and continued through 45 d of spawning. Egg production was impaired by exposure to 3380 μL wsf/L. Tissue residue analyses indicated the uptake of lead, cadmium, and zinc at higher exposure levels. The 30-d LC_{50} for larvae exposed as embryos was >8100 μL wsf/L and for larvae unexposed as embryos was 755 μL wsf/L. The maximum acceptable toxicant concentration for the water-soluble fraction of used crankcase oil was estimated to fall between 325 and 930 μL/L. Based on the compounds identified in the water-soluble fraction, previous toxicity data, and tissue residue analyses, we speculate that zinc, lead, and cadmium probably contribute significantly to the toxicity of the waste oil tested.

Key words: waste oil, used crankcase oil, American flagfish, lethality, reproduction, embryo–larvae, zinc, lead, cadmium

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Des essais de létalité à court terme, essais de survie de 30 d de larves de 1 d et un essai sur le cycle biologique entier démontrent que l'huile de carter usée nuit à la survie et à la reproduction de la jordanelle de floride, *Jordanella floridae*. La CL_{50} après 4 jours de jeunes soumis à des essais en eau dormante et en eau courante sont de 36 200 μL/L et 9500 μL/L, respectivement pour la fraction soluble dans l'eau (wsf) d'un mélange 10% huile–eau et 485

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Printed in Canada (J5071)
Imprimé au Canada (J5071)

μL/L et 82,7 μL/L, respectivement pour les émulsions huile–eau. Sur l'huile utilisée dans les essais et la fraction soluble dans l'eau, nous avons déterminé les caractéristiques descriptives générales de l'huile et la composition en hydrocarbures ainsi que la concentration de 13 métaux. L'essai couvrant le cycle biologique commença avec les embryons et se poursuivit jusqu'à 45 d de ponte. Une exposition à 3380 μL de wsf/L entrave la production d'oeufs. Des analyses de résidus tissulaires indiquent qu'il y a absorption de plomb, de cadmium et de zinc à des niveaux d'exposition plus élevés. La CL_{50} après 30 d de larves exposées comme embryons dépasse >8100 μL de wsf/L, alors que pour des larves non exposées au stade d'embryon est de 755 μL de wsf/L. On estime que la concentration maximale acceptable de toxique pour la fraction soluble dans l'eau d'huile de carter usée se situe entre 325 et 930 μL/L. Nous basant sur les composés identifiés dans la fraction soluble dans l'eau, sur des données de toxicité antérieures et sur des analyses de résidus tissulaires, nous émettons l'hypothèse que le zinc, le plomb et le cadmium contribuent probablement de façon significative à la toxicité de l'huile usée testée.

Received December 12, 1977
Accepted January 18, 1980

Reçu le 12 décembre 1977
Accepté le 18 janvier 1980

POLLUTION by petroleum hydrocarbons has been associated in the past primarily with spectacular marine disasters. However, a significant source of oil spills is the disposal of waste oil in freshwater. Estimates of the waste oil input to the environment in the United States have ranged from 2.27 to 5.03 × 10^9 L yearly (Bernard 1971; Ostrander and Kleinert 1973; Anon. 1974; U.S. Environmental Protection Agency 1974). Approximately 15% of this discharge has been used annually to oil roads for dust control; as much as 70% of this oil is washed off the roadway as runoff (Freestone 1972). In one dust-control spraying incident, the deaths of horses and other animals as well as the illness of several human beings were attributed to toxic components present in the waste oil used (Carter et al. 1975).

In spite of the volume released, freshwater toxicological studies have not been conducted with waste oil. Since both short-term accidental spills and long-term chronic discharges of waste oil can be expected, the purpose of this study was to determine the short-term lethal concentration and the long-term effect of waste oil on the survival, growth, and reproduction of the freshwater American flagfish, *Jordanella floridae*. This fish was chosen because of its rapid development to maturity and the ease of conducting tests on its reproductive success. Four-day lethality tests with both the water-soluble fraction and an oil–water emulsion were conducted to determine potential lethal concentrations due to accidental discharges. Since much aquatic toxicological data are generated in static tests, static as well as flow-through lethality tests were conducted for comparison purposes. It was felt that the majority of long-term environmental exposures would be with the water-soluble fraction. Therefore, long-term tests were conducted with this fraction. Long-term measurements included survival during a 30-d exposure starting with embryos and 1-d-old fish, effects of growth and reproduction of fish exposed from embryos through 45 d of spawning, and 30-d survival of resultant offspring. In addition, the used crankcase oil used as the toxicant was chemically characterized since no previous data on its composition existed.

Materials and Methods

Lake Superior water was used as the dilution water in all tests. Temperatures in flow-through tests were maintained by mixing heated and ambient Lake Superior water, and in static tests by placing all tanks in a constant temperature water bath. All flow-through tests were conducted with a modified 2-L proportional diluter (Mount and Brungs 1967; Benoit and Puglisi 1973).

SHORT-TERM LETHALITY TESTS

Four-day lethality tests were conducted with fish reared in laboratory cultures and fed live nauplii and frozen adult brine shrimp. At the time of testing, all fish had a standard length of 1.6 cm and ranged in age from 30 to 40 d and in weight from 0.13 to 0.14 g. Tests were conducted at 25°C as this has been determined to be suitable for flagfish reproduction (Spehar 1976).

Tests were conducted in all-glass 20 × 30 × 30 cm tanks containing 13.7 L of test solution. Five concentrations plus controls were set up in duplicate with each concentration at one-half of the next highest concentration. The test containers were randomly arranged and in flow-through tests received ~10 tank volumes of toxicant solution per day. A combination of Gro-Lux and Duro-Test[2] fluorescent bulbs was used to provide a constant 16-h daylight photoperiod at 40 ± 51 m at the water surface. Organisms were held at test temperatures at least 2 wk before testing. Feeding was stopped 48 h before beginning a test and was discontinued for its duration. Ten organisms were placed in each container after the addition of oil resulting in loadings of less than 0.1 g/L. In most tests, mortalities were measured at 0, 1, 3, 12, and 24 h and daily thereafter. Death was determined by lack of movement upon gentle prodding, and dead organisms were removed when found. The LC_{50}'s calculated by the moving average angle method (Harris 1959; Finney 1971) were determined only in those tests where the mortality was 65% or higher in one concentration and 35% or lower in another. A test was considered invalid if more than 10% of the control organisms died during a test.

Dissolved oxygen and pH were measured at the beginning, middle, and end of each test; alkalinity and hardness were

[2]The U.S. Environmental Protection Agency neither recommends nor endorses any commercial product; trade names are used only for identification.

measured at the beginning and end (American Public Health Association et al. 1971). In general, the addition of oil caused a slight lowering of pH and dissolved oxygen concentration, but had no effect on hardness or alkalinity. The pH in all tests was between 7.1 and 7.7. The dissolved oxygen concentration was between 60 and 100% saturation in all tests. To prevent the driving off of volatile fractions, none of the tests were aerated. Hardness and alkalinity for all tests ranged from 42 to 49 and 40 to 44 mg/L as $CaCO_3$, respectively.

Life Cycle Exposure

A life cycle toxicity test with *J. floridae* and the water-soluble fraction of used crankcase oil was conducted according to the basic procedures described by Spehar (1976). The life cycle exposure began with embryos and continued through 45 d of spawning by the resultant adults. Duplicate nominal concentrations of 1.0, 0.4, 0.16, 0.06, 0.03, and 0.0% by volume of the water-soluble fraction stock solution were tested. The glass test containers were 30 × 60 × 30 cm for adults, capacity 42.5 L; and 30 × 38 × 30 cm for larvae, capacity 10.6 L. A constant 16-h daylight photoperiod of 100 ± 15 lm measured at the water's surface and a toxicant flow rate of six test-container volumes per day were used.

Tests were initiated by incubating sufficient freshly spawned embryos in each test concentration to get 20 fry for each test chamber to carry through reproductive tests. After 30 d fish were thinned to 15 in each chamber. At 5 wk, two spawning substrates consisting of stainless-steel screen wrapped with blue yarn were placed in each chamber. When a male exhibited territoriality over each substrate, fish were thinned to two males and five or six females. There was occasional female mortality during the test but this appeared to be unrelated to toxicant exposure. At the highest concentration no sexual dimorphism was apparent in one test chamber. The fish in this chamber were thinned to 10 organisms. No sexual maturity was observed in this tank throughout the test.

Reproductive studies were continued for 45 d after initial spawning began. Each day, eggs deposited on spawning substrates were removed, counted, and tested for hatchability. Hatchability was determined by selecting 30–50 embryos from each spawn, placing them in oscillating egg cups, and incubating them in the test chamber until hatching was completed. The number of embryos that hatched was recorded. Fifteen or twenty 1-d-old larvae were then transferred to larvae chambers at the same test concentration. Survival was determined at 4 and 8 d and weekly thereafter.

At the beginning of the life cycle test additional embryos were incubated in each concentration and transferred as 1-d-old larvae to other test concentrations. These tests were run for 30 d to determine if exposure as embryos affected sensitivity as larvae.

Effects of toxicants were determined from changes in survival, growth, eggs produced per female, and egg hatchability. Significant differences were determined by performing an analysis of variance with Dunnett's test of significance ($P < 0.05$; Steele and Torrie 1960).

Metal tissue residues were determined in whole bodies of fish thinned from the life cycle test at various times. A wet digestion procedure followed by atomic absorption spectrophotometry (Leonard 1971) was utilized to measure cadmium, lead, and zinc which were found in high concentrations in the waste oil. Hydrocarbon residues were also measured but the results were inconclusive.

Temperatures were monitored daily and were maintained at 24 ± 1°C with the exception of 1 d during the 2nd wk when temperatures dropped to 18°C for 6 h. Dissolved oxygen concentrations were measured in each test container three times a week and ranged between 65 and 95% saturation in all tanks throughout the test. Hardness, alkalinity, and pH were measured in four test containers each week and ranged from 43 to 48 mg/L as $CaCO_3$, 41 to 45 mg/L as $CaCO_3$, and 7.2 to 7.8 units, respectively. No change due to toxicant concentration was observed.

Toxicant Preparation and Measurement

The waste oil tested was used crankcase oil obtained from the Arrowhead Refinery Company of Duluth, Minnesota. Because waste oils may differ in their hydrocarbon and metal constituents, all oil obtained was from one batch and was stored in sealed oil drums.

Emulsified oils were prepared by blending oil and water at the required ratios in a Waring Blendor for 2 min. In static tests the toxicant solution was prepared at the beginning of the test. For flow-through tests, fresh oil was blended with dilution water each dilution cycle to form a new stock solution each time. This was subsequently dosed into the diluter on the following cycle.

The levels of emulsified waste oil in flow-through tests were monitored by fluorescence as determined on an Aminco-Bowman spectrophotofluorimeter. For each test at least two sets of samples were collected from each concentration plus a duplicate from one test container or from a duplicate concentration. Samples were extracted 1:1 with methylene chloride and, after the aqueous phase was removed, were dried with solvent-washed anhydrous sodium sulfate. The fluorescence of the extract was measured at a peak emission of 360 nm when excited at 300 nm.

The stock solution for the water-soluble fraction was created by recirculating water through an oil layer (10% oil – 90% water) for 18 h and allowing the resutlant mixture to separate for 6 h. Six 45-L glass carboys were connected in series with the water pumped from the bottom of one carboy to the top of the next by a small water pump connected in a line. A *t*-tube was placed on the end of the inlet tube to cause a gentle swirling of the oil and to prevent channeling of the water throughout the oil. After mixing and separation, the water-soluble fraction was drained into a Marriott system for delivery to the diluter. Because of the volume of toxicant required, it was necessary to prepare a new stock solution daily for short-term tests and weekly for the life cycle test.

The percentage of dilution of the used crankcase oil water-soluble fraction in flow-through tests was monitored by measuring lead with flameless atomic absorption spectrophotometry. Lead is a major contaminant of used crankcase oil because of the organometallic additives in lubricating oil as well as that picked up during engine wear (Supp et al. 1973; U.S. Environmental Protection Agency 1974). By measuring lead in the test chambers and in the water-soluble fraction stock solution, it was possible to determine the percentage of dilution of the water-soluble fraction present in the test containers.

Twice weekly, samples for lead analysis from each test container and the stock solution were collected in polyethylene bottles from mid-depth and preserved with concentrated perchloric acid. At least once a week duplicate samples were collected from a randomly selected container.

TABLE 1. Concentrations of the water-soluble fraction of used crankcase oil determined from measured lead concentrations used in test containers for the *Jordanella floridae* life cycle test.

Measured lead concn. $\bar{X} \pm$ SD (μg/L)	Range (μg/L)	No. of analyses	μL/L stock solution based on $\bar{X}$ lead concn. (avg.)
37.0±8.3[a]	22.7–56.0	23	7920
38.7±8.5	24.8–55.1	21	8290 (8100)
16.0±3.9	7.33–23.4	22	3430
15.5±4.2	7.22–23.8	30	3320 (3380)
4.50±1.94	2.68–11.8	22	960
4.22±1.47	2.52–8.42	21	900 (930)
1.56±0.47	1.03–2.65	21	330
1.48±0.35	0.98–2.54	20	320 (325)
0.77±0.34	0.30–1.97	22	160
0.71±0.24	0.32–1.29	21	150 (155)
0.19±0.19	0.01–0.64	23	—
0.15±0.14	0.01–0.60	21	—
Stock solution 4670±750	3440–6610	30	

[a]Each pair represents duplicate concentrations.

Measured levels for the life cycle test are reported in Table 1.

In all tests recovery of lead-spiked samples compared to aqueous standards was 90% or greater, replicate samples from the same test container agreed 95% or greater, and duplicate test containers agreed 90% or greater.

Characterization of the Waste Oil and its Water-Soluble Fraction

Because little previous knowledge on the composition of the waste oil tested was available, the used crankcase oil and the water-soluble fraction of that oil were analyzed. The organic components of the crankcase oil and its water-soluble fraction were characterized by separating the hydrocarbons from water with XAD-2 resin columns and subsequently into volatiles, saturated hydrocarbons, aromatic hydrocarbons, polar compounds, and solids by means of a silicic acid column. Analyses were conducted with gas chromatography mass spectrometry (GC/MS) according to methods previously described by Hedtke et al. (1974). The water-soluble fraction was also examined by gas–liquid chromatography (GLC) to estimate total saturated hydrocarbon concentration. Using the saturated hydrocarbon fraction separated from the waste oil by a silicic acid column as a standard, the concentration of total saturated hydrocarbons in the water-soluble fraction stock solution was determined. Additional analyses on the crankcase oil were performed by the Bartlesville Energy Research Center, U.S. Bureau of Mines. Included were general descriptive characteristics of lubricating oils and 13 metals. Metal concentrations in the water-soluble fraction were measured by atomic absorption again as described by Hedtke et al. (1974).

Results and Discussion

Toxicant Characterization

The chemical characterization of the crankcase oil and its water-soluble fraction (Table 2) indicated the oil was generally similar to eight other used lubricating oils analyzed by the U.S. Bureau of Mines (M. L. Whisman, Bartlesville. Energy Research Center, Bartlesville, OK 74003, 1973, unpublished data). Exceptions were a slightly lower calcium concentration and flash point and high percentage of sulphur and

TABLE 2. Characteristics of the used crankcase oil and its water-soluble fraction.

Item	Oil	Water-soluble fraction[a]
Inspection tests for lubrication units		
Sp gr (15.6/15.6°C)	0.906	—[b]
Viscosity		
SUS 37.8°C	290	—
SUS 99°C	60.9	—
Viscosity index	170	—
Carbon residue (%)	4.3	—
Flash point (°C)	58.5	—
Nitrogen (%)	0.090	—
Sulfur (%)	0.54	—
Antifreeze (%)	<0.1	—
Saponification number	14.5	—
Gasoline dilution	3.2	—
Total acid number	5.91	—
Total base number	2.06	—
Pentane insolubles	3.2	—
Coagulated pentane insolubles (%)	11.1	—
Benzene insoluble	1.45	—
Coagulated benzene insoluble	1.85	—
Solids (%)	15.6	—
Metals (μg/g for oil, μg/L for water-soluble fraction)		
Aluminum	15	<100
Copper	18	<100
Iron	220	<100
Lead	18 500	4 400±200[c]
Nickel	—	<100
Silicon	17	—
Antimony	6	—
Vanadium	—	—
Sodium	59	—
Barium	177	—
Calcium	688	—
Zinc	1 360	16 000±1000
Magnesium	410	—
Mercury	—	<0.1
Cadmium[d]	—	60±2
Molybdenum[d]	—	<200
Cobalt[d]	—	0.4±0.4
Arsenic[d]	—	<300
Selenium[d]	—	<100
Chromium	—	<140
Manganese	—	<100

TABLE 2 (*Concluded*)

Item	Oil	Water-soluble fraction[a]
Organics (+ indicates present but not quantified)		
Saturated hydrocarbons (%)	76.6	3.2
Aromatic hydrocarbons (%)	4.1	14.5
C_2 alkyl benzene	—	+
C_3 alkyl benzene	+	+
C_4 alkyl benzene	+	+
C_5 alkyl benzene	+	+
Ethylbenzene	+	—
Naphthalene	+	+
Methylnaphthalene	+	+
C_2 alkyl naphthalene	+	—
C_3 alkyl naphthalene	+	—
Phenanthrene	+	—
Methylphenanthrene	+	—
C_2 alkyl phenanthrene	+	—
C_3 alkyl phenanthrene	+	—
Dihydrodimethylphenanthrene	+	—
Dihydrodimethylindene	+	—
Diphenylmethane	+	—
Ethylstyrene	+	—
Dimethylindene	—	+
Polar compounds (%)	3.8	82.0
Volatile hydrocarbons (%)	3.2	—
C_2 alkyl benzene	+	—
C_3 alkyl benzene	+	—
C_4 alkyl benzene	+	—
C_5 alkyl benzene	+	—
Naphthalene	+	—
Methylnaphthalene	+	—
C_2 alkyl naphthalene	+	—

[a]Analysis of one test solution only. Lead concentrations in solutions prepared weekly for 30 wk were 4670 ± 750.
[b]Analyses not conducted or none detected.
[c]Mean multiple analyses ± standard deviation.
[d]Does not necessarily include contribution from a highly volatile fraction.

lead concentration. In the water-soluble fraction there was 3.2% saturated hydrocarbons compared to 76% in the oil and 14.5% aromatics compared to 4.1% in the oil. The concentration of total saturated hydrocarbons in the water-soluble fraction was found to be 11.6 mg/L. No chlorinated compounds were found. The presence of organo-lead compounds was suspected because of the removal of greater than 90% of the lead in the water-soluble fraction by the XAD column. More than 90% of the lead, zinc, and cadmium was in the dissolved fraction, which was operationally defined as those components passing through a 0.45-μm membrane filter.

SHORT-TERM LETHALITY TESTS

The calculated 96-h LC_{50}'s (and confidence intervals) for the water-soluble fraction (wsf) of waste oil in static and flow-through tests were 36 200 μL wsf/L (27 700 – 47 700 wsf/L) and 9500 μL wsf/L (6400–17 800 μL wsf/L), respectively. Ninety-six-hour LC_{50}'s for oil–water emulsions in static and flow-through tests were 485 μL oil/L (no confidence interval possible) and 82.7 μL oil/L (64.9–110 μL oil/L), respectively.

The short-term lethality tests indicated that flow-through tests were more toxic than static tests and emulsified oil was more toxic than the water-soluble fraction. Static tests were likely to be less toxic than flow-through due to the changes in chemical nature and toxicity of oil with time. Oils modified by aging have been shown to be less toxic than fresh oil (Morrow 1973; Templeton et al. 1975). In the flow-through tests, fresh oil was intermittently added throughout the test. The increased toxicity of emulsified oils could have been caused by increased oil in the water column, by a coating of the gills, and by a more rapid release of the soluble components due to the increased surface area for oil–water contact.

The greater toxicity of emulsified oils suggests that if long-term tests had been conducted with oil–water emulsions, effects might have been found at lower levels than with the water-soluble fraction. As stated previously, however, it was felt that while emulsions are likely to occur on a short-term basis, long-term exposures are more likely with the water-soluble fraction.

LONG-TERM TESTS

Exposure of flagfish to the water-soluble fraction of used crankcase oil during the life cycle test resulted in significantly ($P < 0.05$) lower egg production per female at 3380 μL wsf/L (Table 3). No difference was found at 930 μL wsf/L. In general, growth and embryo hatchability were not affected by exposure to oil at the concentrations tested. In addition, there was no significant larval mortality at any concentration when hatched and exposed at the same concentration (Table 4); the 30-d LC_{50} was greater than 8110 μL wsf/L. However, for those larvae hatched in control water and transferred as 1-d-old fish to various exposure concentrations, the 4-, 8-, and 30-d LC_{50}'s calculated from the data in Table 4 were 1660, 1000, and 755 μL wsf/L, respectively. No statistically significant toxicant related difference ($P < 0.01$) was found between the 30-d survival of second-generation larvae which were spawned, hatched, and exposed to the same concentrations at their respective parental exposure levels where spawning was adequate for testing. Mean survival ranged from 62.5 to 70.4%. No tests were possible at 8110 μL/L since spawning was insufficient to obtain adequate embryos for testing.

Whole body tissue residue levels for lead, cadmium, and zinc were higher at 3380 μL/L and 8110 μL/L than at 155 μL/L and in the controls (Table 5). There was little difference in residues at the two sampling time intervals. Statistical treatment of the data was not possible because of the limited number of replicate fish available for analyses.

TABLE 3. Growth and reproduction of *Jordanella floridae* exposed to the water-soluble fraction of used crankcase oil.

Mean oil concentration (μL water-soluble fraction/L water)	Females[a]			Males			Total eggs produced	Eggs per female (total/no. females)	Hatchability		
	No. present	Mean weight (g)	Mean length (cm)	No. present	Mean weight (g)	Mean length (cm)			Mean % hatch	Range % hatch	No. of tests
8100	4[b]	0.43[c]	2.4[c]	4[b]	0.98	3.0	3	<1[c]	—	—	0
	5	0.86	3.1	2	1.31	3.4	53	11[c]	—	—	0
3380	4	0.85	2.8	3	0.86	3.0	110	27[c]	67	67	1
	4	1.23	3.2	2	2.92	4.5	5	1[c]	—	—	0
930	5	0.93	3.1	2	2.11	4.1	973	195	58.3	28–100	9
	3	0.86	3.0	2	2.01	4.0	603	201	61.7	43–76	3
325	5	1.08	3.2	2	1.61	3.7	2070	415	60.4	46–89	15
	5	1.44	3.5	2	1.50	3.6	1420	285	59.0	32–82	9
155	6	1.02	3.2	2	1.77	4.0	1070	179	68.6	38–92	11
	5	0.90	3.1	2	1.97	3.9	827	165	57.3	38–76	6
0 (control)	6	1.10	3.4	2	2.15	3.8	1960	326	46.5	18–96	8
	5	0.96	3.2	2	2.14	4.2	1160	232	50.8	28–76	5

[a]Each pair represents duplicate tanks.
[b]Fish never reached sexual maturity.
[c]Significantly different from control value $P < 0.05$.

The long-term effects on survival and reproduction occurred at substantially lower concentrations than in the short-term lethality tests with juvenile fish. The lowest concentration affecting reproduction was 3380 μL wsf/L. The most sensitive test was the 30-d survival of larvae unexposed as embryos. The 30-d survival of larvae unexposed as embryos was significantly lower at 930 μL wsf/L (47.5% survival). The maximum acceptable toxicant concentration (MATC, Mount and Stephan 1967) or the highest concentration where there was no affect on survival, growth, or reproduction for the water-soluble fraction of a 10% mixture of used crankcase oil and water was, therefore, between 325 and 930 μL wsf/L.

The sensitivity of the embryo–larvae stage has been extensively reviewed previously (McKim 1977). Of the 56 tests included in this review, 82% of the time embryo–larvae or early juvenile exposures were identical with, or more sensitive than, other life cycle effects. The author states that while most of the data involves single toxicants, complex mixtures should give similar results. In the present test, larvae exposed as embryos

TABLE 4. Percentage survival of 1-d-old *Jordanella floridae* at various concentrations of the water-soluble fraction of used crankcase oil.

Embryo exposure (μL water-soluble fraction/L)	Larval exposure (μL water-soluble fraction/L)	Mean percentage survival[a]		
		4 d	8 d	30 d
0 (control)	8110	0.0	0.0	0.0
	3380	13.4	3.4	3.4
	930	81.7	70.9	47.5
	325	100.0	90.0	86.7
	155	92.5	90.0	90.0
	0	100.0 (4)	91.7 (4)	88.8 (4)
155	3380	0.0	0.0	0.0
	155	100.0	97.5	97.5
325	8110	2.5	2.5	2.5
	3380	—[b]	—	40.0
	325	100.0	97.5	95.0
930	8110	86.7 (1)	86.7 (1)	86.7 (1)
	3380	100.0 (1)	100.0 (1)	100.0 (1)
	930	100.0	97.5	85.0
	155	100.0	95.0	95.0
3380	8110	100.0 (1)	100.0 (1)	95.0 (1)
	3380	100.0	92.5	92.5
	325	97.5	92.5	92.5
8110	8110	97.5	82.5	82.5
	930	100.0	97.5	97.5
	0	100.0	97.5	92.5

[a]Mean of two tests with 15 fish per test unless number of tests otherwise indicated in parentheses.
[b]Observation not made.

TABLE 5. Metal residues in whole *Jordanella floridae* tissues after life cycle exposure to used crankcase oil.

Oil concn. (μL water-soluble fraction/L)	Exposure time (days)	Metal residue[a] (μg/g dry weight)		
		Lead	Cadmium	Zinc
0 (control)	30	<1.3	0.11	119
	75	1.4[b]	0.30[b]	99[b]
155	30	1	0.15	146
	75	1.3[b]	—	102[b]
3380	30	25	1.1	187
	57	22	0.9	143
8110	30	40	1.2	256
	64	50	1.3	291

[a]Average of at least duplicates of a two-fish composite.
[b]Based on a single two-fish composite.

were not affected by the waste oil concentrations tested and, therefore, were not as sensitive as the reproductive stage of the life cycle. However, tests with larvae not exposed as embryos were more sensitive than any other test. Thus, the evaluation of the sensitivity of embryo–larval tests may be affected by the stage of development during initial toxicant exposure.

The difference in 30-d LC_{50}'s between larvae exposed as embryos (>8110 μL wsf/L) and unexposed as embryos (755 μL wsf/L) suggests that acclimation occurred during the embryo stage. Additional evidence of acclimation was found with larvae exposed to oil at various concentrations as embryos and transferred upon hatching to other concentrations. At the lower concentrations (155 and 325 μL/L) the acclimation to waste oil allowed larval survival at the hatching concentrations. However, substantial mortality occurred when larvae were exposed to higher concentrations. Larvae exposed as embryos to concentrations of 930 μL/L and higher survived for 30 d at all tested concentrations. This implies an embryo-exposure threshold for acclimation to the range of concentrations tested. Other studies with zinc have also found acclimation due to exposure during the embryo stage (Pickering and Vigor 1965; Singley et al. 1974; Spehar 1976; Spehar et al. 1978). This apparent acclimation during the embryo stage may also have affected other end points of effect. Other life cycle effects might have occurred at lower concentrations than those found if the test started with 1-d-old larvae rather than embryos. Spehar (1976) found with zinc that the growth of female flagfish was affected at lower levels when started as 1-d-old larvae compared to those started as embryos.

A review of the identified compounds in the water-soluble fraction, previous toxicity data, and tissue-residue analysis suggests that the metals zinc, lead, and cadmium probably contribute significantly to the toxicity in the tests reported here. A decrease in embryo production by *J. floridae* in soft water has been reported by Spehar (1976) at concentrations of zinc between 139 and 267 μg/L. The 30-d LC_{50}s calculated from Spehar's data were 64 μg/L for fry not exposed as eggs and 171 μg/L for fry exposed as eggs. Concentrations of up to 180 μg/L zinc were found in the higher oil concentrations in the present study. Cadmium concentrations of 8 μg/L (Spehar 1976) and lead concentrations of 62.5 μg/L (L. Anderson, U.S. Environmental Protection Agency, Environmental Research Laboratory—Duluth, 1976, personal communication) reduced flagfish reproduction in soft water. The highest tested oil concentration contained 0.5 μg/L cadmium and 38 μg/L lead. The effect of combinations of all three of these metals, however, has not been determined. In addition, the toxicity of organometallic compounds is largely unknown. From a life cycle test with *Pimephales promelas* and copper, zinc, and cadmium in hard water, Eaton (1973) concluded that the toxicities of the individual components were not strictly additive when mixed. Spehar et al. (1978) found that the toxicity of a mixture of cadmium and zinc to flagfish egg production was greater than for the individual metals but statistical significance could not be demonstrated. Effects on larval survival were attributed primarily to zinc in that same study. It is likely that the long-term effects of used crankcase oil are a result of a combination of a number of the components of the oil.

Acknowledgments

We thank Dr G. D. Veith, Dr J. E. Poldoski, and Mr D. W. Kuehl for their characterization of the oil and analyses of the fish tissues. We also thank Mr L. F. Mueller for his assistance in conducting these tests and Ms B. J. Halligan for performing routine chemical analyses.

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